

WHAT IS CLAIMED IS:

1. A method of isolating an HIV capsid intermediate, said method comprising the steps of:
5 combining HIV Gag Pr55 mRNA with a cell-free protein translation mixture containing myristoyl coenzyme A;
incubating said translation mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into immature HIV capsids;
identifying said capsid intermediates on a linear sucrose gradient; and
10 isolating said capsid intermediates by immunoprecipitation with antibodies specific for HIV Gag, whereby isolated capsid intermediates are obtained.
2. The method according to Claim 1, wherein the amount of said myristoyl coenzyme A is about 0.1 to 100 micromolar.
3. The method according to Claim 1, wherein said cell-free extract contains a detergent sensitive fraction from eukaryotic cells.
4. The method according to Claim 1, wherein said cell-free extract contains a detergent-insensitive fraction from eukaryotic cells.
5. The method according to claim 1, wherein said cell-free extract contains an ATP sensitive fraction from eukaryotic cells.
- 25 6. A method of isolating an HIV capsid intermediate, said method comprising the steps of:
combining HIV Gag Pr55 mRNA with a cell-free protein translation mixture containing myristol coenzyme A present in a concentration ranging from about 0.1 to 100 micromolar, wherein said cell free mixture contains a detergent-insensitive or a
30 detergent sensitive fraction from eukaryotic cells;
incubating said translation mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into immature HIV capsids;
identifying said capsid intermediates on a linear sucrose gradient; and
isolating said capsid intermediates by immunoprecipitation with antibodies specific
35 for HIV Gag, whereby isolated capsid intermediates are obtained.

7. A method of identifying chaperone proteins involved in HIV capsid assembly, said method comprising the steps of:
denaturing affinity purified capsid intermediate complexes so that said complexes are separated into chaperone proteins and capsid proteins;
5 removing separated capsid proteins with monoclonal antibodies specific for capsid proteins leaving a mixture of chaperone proteins;
isolating individual chaperone proteins from said mixture;
sequencing said individual chaperone proteins, and
comparing the sequences of said individual chaperone proteins to known sequences of
10 host proteins, whereby the identity of host proteins that are involved in HIV capsid assembly are obtained.
8. A method of identifying chaperone proteins bound to HIV capsid intermediates produced in a mammalian cell, said method comprising the steps of:
15 sequencing a human homologue to said chaperone protein identified in the cell-free system according to claim 7 using degenerate primers;
expressing said human homologue in the cell-free system that has been immunodepleted for cell-free chaperone proteins wherein said sequence of human homologue is cloned into an expression vector; and
20 measuring capsid formation in a cell-free translation system programmed with HIV Gag compared to a cell-free translation system programmed with HIV Gag that has not been immunodepleted, whereby comparable amounts of HIV capsid formation identifies chaperone protein produced in mammalian cells that bind to HIV capsid intermediates.
9. A method of identifying chaperone proteins bound to HIV capsid intermediates produced in a mammalian cell, said method comprising the steps of:
sequencing a human homologue to said chaperone protein identified in the cell-free
25 system according to claim 7 using degenerate primers;
30 expressing said human homologue in HIV infected mammalian cells that are stably transfected with a dominant negative HP68 mutant wherein said sequence of human homologue is cloned into an expression vector; and
measuring HIV viral release from said cells compared to cells not stably transfected with a dominant negative HP68 mutant, whereby restoration of HIV viral release
35 identifies chaperone proteins produced in mammalian cells that bind to HIV capsid intermediates.

10 A method of identifying conformers of host chaperone proteins that bind to HIV
capsid intermediates, said method comprising the steps of:
contacting isolated host proteins having an amino acid sequence substantially similar
5 to a host chaperone protein with a plurality of monoclonal antibodies that bind to said
host protein;
identifying from said plurality of monoclonal antibodies those that bind to a subset of
said host proteins and do not bind to said host chaperone proteins;
isolating said host cell proteins so identified; and
10 determining whether said conformer functions to facilitate assembly of HIV capsid
intermediates, whereby conformers that so function are identified as conformers of
said host chaperone protein.

11. A method of identifying a functional HP68 conformer, said method comprising the
steps of:
5 (a) isolating an RNase L inhibitor in cells not infected with HIV that does not bind to
HIV Gag;
(b) isolating HP68 that binds HIV Gag in cells producing HIV Gag;
(c) expressing said RNase L inhibitor in a cell-free translation system that has been
immunodepleted for HP68, is programmed with HIV Gag mRNA and comprises
20 an expression vector comprising a nucleic acid sequence encoding said RNase L
inhibitor;
(d) expressing said HP68 in a cell-free translation system that has been
immunodepleted for HP68, is programmed with HIV Gag mRNA and comprises
25 an expression vector comprising a nucleic acid sequence encoding said HP68; and
(e) comparing capsid formation in step (c) to capsid formation in step (d), whereby a
difference in amount of HIV capsids produced is indicative of a conformer of
HP68 specific for HIV capsid formation.

30 12. A method of producing monoclonal antibodies to a conformer of a host protein that is
involved in assembly of immature HIV capsids, said method comprising the steps of:
immunizing knockout mice with a host chaperone protein, wherein said knockout
mice have a non-functional gene that no longer codes for said conformer and lack the
ability to produce said protein;
35 producing hybridoma cells from spleens of said mice;
screening said hybridoma cells for production of antibodies to both a native and a

denatured conformer of said host chaperone protein; and
propagating hybridoma cells producing antibodies that bind substantially specifically
to said host chaperone protein and not to conformers of said host chaperone protein
that do not bind Gag and do not facilitate HIV capsid assembly, whereby antibodies to
5 native and denatured said protein or peptide conformer of interest are produced.

13. Monoclonal antibodies produced according to the method of Claim 12.

14. Binding fragments to said conformer derived from monoclonal antibodies produced
10 according to the method of Claim 12.

15. A method of identifying a binding site between a host chaperone protein and an
intermediate in HIV capsid assembly, said method comprising the steps of:
obtaining a conformational epitope map of said host protein using monoclonal
5 antibodies produced according to the method Claim 12;
obtaining a conformational epitope map of HIV Gag using monoclonal antibodies
specific for Gag;
obtaining a conformational epitope map of HIV capsid intermediates using
monoclonal antibodies specific for HIV Gag and antibodies produced according to the
20 method of Claim 12; and
comparing conformational epitope maps of HIV Gag, capsid intermediate complex
and said conformer, whereby the binding site on said conformer for HIV Gag is
identified and the binding site on HIV Gag for said conformer is identified.

16. A method for identifying compounds that interfere with HIV capsid assembly by
specifically binding to and preventing said conformer from binding to HIV Gag, said
method comprising the steps of:
(a) screening databases for compounds that bind to the binding site identified
30 according to the method of Claim 15, whereby potential compounds are obtained;
(c) screening said potential compounds for test compounds that bind substantially
specifically to a host chaperone protein for HIV capsid assembly but not to
conformers of said host chaperone proteins that do not bind HIV Gag;

(d) screening said test compounds in a cell free translation system, wherein efficacy is measured by a decrease in HIV capsid production; and

(f) further screening said compounds for ability to block HIV capsid formation in mammalian cells infected with HIV, whereby compounds that block HIV capsid formation are identified.

17. A method for identifying compounds that interfere with HIV capsid assembly by specifically binding to and preventing said conformer from binding to HIV Gag, said method comprising the steps of:

- a) expressing HIV Gag in a mammalian cell;
- b) identification of co-localization of HIV Gag and HP68 using immunofluorescence in said mammalian cells, and;
- c) screening said potential compounds for test compounds that interfere with co-localization of HP68 and Gag in said mammalian cells whereby compounds that interfere with HIV capsid assembly are identified by a diffuse staining pattern of HP68.

18. The method according to the method of Claim 17, wherein said compounds do not cause toxicity or upregulate host stress proteins in said mammalian cells.

19. A method of identifying a binding site between a host chaperone protein and an intermediate in HIV capsid assembly, said method comprising the steps of:
obtaining a conformational epitope map of said host protein using monoclonal antibodies produced according to the method Claim 12;
obtaining a conformational epitope map of HIV Vif using monoclonal antibodies specific for Vif;
obtaining a conformational epitope map of HIV capsid intermediates using monoclonal antibodies specific for HIV Vif and antibodies produced according to the method of Claim 12; and
comparing conformational epitope maps of HIV Vif, capsid intermediate complex and said conformer, whereby the binding site on said conformer for HIV Vif is identified and the binding site on HIV Vif for said conformer is identified.

20. A method for identifying compounds that interfere with HIV capsid assembly by specifically binding to and preventing said conformer from binding to HIV Vif, said method comprising the steps of:
- 5 (a) screening databases for compounds that bind to the binding site identified according to the method of Claim 15, whereby potential compounds are obtained;
- (c) screening said potential compounds for test compounds that bind substantially specifically to a host chaperone protein for HIV capsid assembly but not to conformers of said host chaperone proteins that do not bind HIV Vif;
- 10 (d) screening said test compounds in a cell free translation system, wherein efficacy is measured by a decrease in HIV capsid production; and
- (g) further screening said compounds for ability to block HIV capsid formation in mammalian cells infected with HIV, whereby compounds that block HIV capsid formation are identified.
- 5 21. The method according to the method of Claim 20, wherein said compounds do not cause toxicity or upregulate host stress proteins in said mammalian cells.
22. A method for establishing a profile of host protein HIV capsid assembly chaperones and their conformers in a population of individuals infected with HIV, wherein said profile is relative to specific HIV characteristics, said method comprising the steps of:
- 20 compiling a conformer profile of host protein HIV capsid assembly chaperones and their conformers in individual members of said population, wherein said individual members produce HIV virions; and
- establishing a relationship between said conformer profiles of said individual members and specific characteristics of HIV in said individual members, whereby a
- 25 population profile of conformers relative to specific HIV characteristics is obtained.
23. A method for selecting a treatment to administer to a individual infected with HIV, said method comprising the steps of:
- 30 determining a conformer profile of host protein HIV capsid assembly chaperones and their conformers of said individual;
- comparing said conformer profile of said patient to a conformer population profile obtained according to the method of Claim 22; and

selecting as a method of treatment for said individual a method of treatment that was successful for treatment of individual members of said population having a substantially similar conformer profile, whereby a treatment based on a conformer profile is selected for said individual.

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24. HIV capsid intermediates produced by a cell free system comprising:

a) HIV Gag Pr55 mRNA;

b) cell-free extract, amino acids, transfer RNA (tRNA), ribosomes and an energy source;

10 c) a concentration of myristoyl coenzyme A about 0.1 to 100 micromolar;
wherein said capsid intermediates are selected from the group consisting of proteins having a buoyant density of about 10S, about 80S, about 150S and about 500S.

15 25. The HIV capsid intermediates produced according to Claim 24, wherein said intermediates comprise HIV capsid proteins and host chaperone proteins.

26. The HIV capsid intermediates produced according to Claim 24, wherein said intermediates comprise Gag and HP68.

20 27. The HIV capsid intermediates produced according to Claim 24, wherein said intermediates comprise Vif and HP68.

28. The HIV capsid intermediates produced according to Claim 24, wherein said intermediates comprise HP68 that binds to Gag and Vif but does not bind to RNase L.

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29. A cell-free system for translation and assembly of an HIV capsid, comprising a cell-free translation mixture, an mRNA molecule encoding a Gag Pr55 protein derived from human immunodeficiency virus (HIV), and myristoyl coenzyme A.

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30. The cell-free translation system of claim 29, which further includes a detergent-sensitive fraction derived from eukaryotic cell membranes.

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31. The cell-free translation system of claim 29, which further includes a eukaryotic cell component characterized by insensitivity to a concentration of at least 0.5% (wt/vol) “NIKKOL” detergent.
32. The cell-free translation system of claim 29, wherein said system further includes HIV genomic RNA or a fragment thereof.
- 10 33. The cell-free translation system of claim 29, which further includes (i) a DNA molecule which encodes HIV Gag Pr55, (ii) an RNA polymerase for synthesizing said mRNA, and (iii) sufficient concentrations of nucleotides ATP, UTP, GTP, and CTP to support such mRNA synthesis.
- 15 34. The cell-free translation system of claim 29, wherein said HIV Gag mRNA encodes a mutant defective in assembly.
- 20 35. A method of producing an HIV capsid intermediate in a cell-free system, comprising adding to a cell-free protein translation mixture which contains a cell-free extract, amino acids, transfer RNA (tRNA), ribosomes and an energy source: (i) an mRNA molecule encoding an HIV Pr55 Gag protein, and (ii) a concentration of myristoyl coenzyme A that is greater than about 0.1 micromolar, to form a reaction mixture; incubating said reaction mixture for a period of time sufficient to assemble Gag Pr55 mRNA translation products into an immature HIV capsid.
- 25 36. The method of claim 35, wherein said reaction mixture is supplemented with (iii) a detergent-sensitive fraction derived from eukaryotic cell membranes, and (iv) a eukaryotic cell component characterized by insensitivity to a concentration of at least 0.5% (wt/vol) “NIKKOL” detergent.
- 30 37. The method of claim 35, wherein said reaction mixture is supplemented with host protein HP 68 or a homolog thereof.
- 35 38. The method of claim 35, which further includes adding to said reaction mixture an HIV genomic RNA molecule or a fragment thereof.
39. The method of claim 35, which further includes adding to said reaction mixture a Gag

Pr55 DNA transcript and a transcription mixture containing an RNA polymerase and ribonucleotides ATP, UTP, GTP and CTP effective to produce said Gag mRNA in said cell-free mixture.

- 5 40. An isolated HIV capsid intermediate selected from a group of HIV capsid intermediates having buoyant densities selected from the group of about 10 S, about 80 S, about 150 S and about 500 S, wherein said buoyant densities are measured by sedimentation in a linear sucrose density gradient ranging from 15% to 60% sucrose.
- 10 41. A method of selecting a compound capable of altering HIV capsid assembly, comprising
adding a test compound to a reaction mixture which includes (i) a cell-free translation mixture that includes a cell-free extract, tRNA, ribosomes, amino acids and an energy source, (ii) an mRNA molecule encoding HIV Gag Pr55, (iii) myristoyl coenzyme A,
15 present at a concentration greater than about 0.1 micromolar,
measuring capsid assembly in the presence of said test compound,
comparing assembly in the absence of said test compound to assembly in the presence of said compound,
selecting the compound as a compound capable of altering HIV capsid assembly if
20 assembly measured in the absence of said compound is significantly different than assembly measured in the presence of said compound.
42. The method of claim 41, wherein said measuring of capsid assembly includes measuring formation of assembly intermediates.
- 25 43. A host cell protein, comprising:
a peptide region having the sequence presented as SEQ ID NO: 2,
specific immunoreactivity with monoclonal antibody 23 c, and
an apparent molecular weight of about 68 kilodaltons,
30 wherein said protein associates with HIV capsid intermediates produced by the cell-free translation system of claim 29.
44. The host cell protein of claim 43, wherein said protein is characterized by at least 75% amino acid sequence identity to HP 68.
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45. The host cell protein of claim 43, which is derived from wheat germ extract and which is identified as HP 68.
46. A method of inhibiting HIV capsid formation in a cell, comprising
5 adding to the cell a compound selected for its ability to inhibit HIV capsid formation in a cell-free translation system consisting essentially of (i) a cell-free translation mixture which contains a cell-free extract, tRNA, ribosomes, amino acids and an energy source, (ii) an mRNA molecule encoding a HIV capsid assembly protein Pr55, and (iii) myristoyl coenzyme A.
- 10 47. The method of claim 46, wherein said compound is selected for its ability to block association of the host protein of claim 41 or sequence homologs thereof with an HIV capsid intermediate.
- 15 48. A method of selecting a compound capable of altering HIV capsid assembly in cells, comprising
adding a test compound to cells that are forming retroviral capsids,
measuring the quantity and nature of capsid assembly intermediates formed within
cells in the presence of said test compound,
20 comparing the quantity and nature of assembly intermediates formed within cells in the absence of said test compound to said quantity and nature of intermediates formed in the presence of test compound,
selecting the compound as a compound capable of altering formation of HIV
assembly intermediates if the quantity or nature of intermediates measured in the
25 presence of said compound is significantly different than the quantity or nature of intermediates measured in the absence of said compound.
- 30 49. The method of claim 48, wherein said selected retrovirus is HIV, and said measuring of said capsid formation is accomplished by measuring association of HP68 or a homolog thereof with an HIV capsid intermediate.
50. A method for encapsidating genomic HIV RNA or fragments thereof, comprising
adding said RNA or RNA fragments to a cell-free translation system as defined in
claim 29, and
35 incubating said system for a period of time sufficient to complete said translation and

assembly reaction.